In vitro placental self and cross pollination in some species

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Abstract

Excised placentae with ovules of *Primula pubescens*, *P. auricula*, *Scopolia carniolica*, *Digitalis purpurea*, *Torenia fournieri* and *Chionodoxa luciliae* were self pollinated *in vitro* and the development of seeds was observed. The same method was used for obtaining hybrid globular embryos from crosses between: *P. pubescens* × *P. auricula*, *Scopolia carniolica* × *Physochlaina praealta*, *Melandrium album* × *Silene saxifraga* and *M. album* × *Arenaria pungens*.

Key words: Pollination *in vitro*, embryos

INTRODUCTION

The technique of *in vitro* pollination and fertilization presents new possibilities for solving some problems involved in pollination, fertilization and the development of embryos. This technique makes it possible to: a) obtain viable seeds capable of germination *in situ*; b) raise interspecific and intergeneric hybrid embryos and plants; c) bypass self incompatibility when the barriers of pollen tube growth lie in the stigma and style; d) induce parthenogenesis and the development of haploid embryos (Rangaswamy and Shivanna 1967, Niimi. Wagner and Hess 1973, Stewart 1981, Zenkteler 1967, 1970, 1980). In spite of the fact that fertilization of ovules *in vitro* has been a functional technique for years, the number of species which have been reproduced sexually *in vitro* is still very small (Zenkteler 1990). Therefore, the aim of the present study was: a) to investigate the processes of

fertilization and embryo development in 6 species representing 5 families after self pollination of ovules; b) to perform interspecific and intergeneric pollination of ovules among 7 species representing 3 families.

MATERIAL AND METHODS

Flower buds were bagged two days before anthesis. Pistils were briefly surface sterilized with 70% ethanol, followed by 10-15 min in chlorine water and rinsed 3 times in sterile water. Later the style and the ovary wall were removed and the placentae with the ovules were inoculated on the medium prescribed by Nitsch (1951). The same day the closed flower buds were harvested 24-48 hours before natural dehiscence. Anthers were dissected from the buds and kept in sterile Petri dishes until they dehisced. Using a sterile scalpel, pollen grains were placed directly on the surface of the exposed ovules. All experimental materials were cultured under diffuse light at a temperature of 22-26°C. For embryological and cytological investigations the enlarged ovules were fixed in Carnoy's solution, passed through an alcohol-xylene series and embedded in paraffin. 12- μ m sections were stained with crystal violet and orange G.

RESULTS

SELF-POLLINATION OF OVULES

Primula pubescens. Pollen grains germinated abundantly, fertilization took place two days following pollination and the ovules matured into seeds within 5 weeks. Mature embryos were isolated and cultured on MS medium (Murashige and Skoog 1962) on which after one week of culture they developed into seedlings (Fig. 1).

Primula auricula. Six days after pollination many ovules enlarged (Fig. 2). Microscopical analysis of these ovules revealed the presence of normally developed globular embryos and endosperm. Young seeds containing globular embryos were still present during the next 2 weeks of culture and the embryos did not develop further.

Scopolia carniolica. Fertilization took place one day following pollination. Fertilized ovules gradually increased in size and matured into seeds within 3 weeks (Fig. 3). Embryos at the torpedo stage were isolated and transferred to a fresh MS medium on which a few days later grew into seedlings (Fig. 4).

Digitalis purpurea. Excised placentae were pollinated on the next day after inoculation. The pollen grains started to germinate after several hours. Some ovules increased in size and 27 days after pollination seeds were formed. The seeds contained embryos at different stages of development, usually at the

globular stage (Fig. 6). however, in some seeds embryos at the torpedo stage were observed (Fig. 5). The torpedo embryos were isolated and cultured on MS medium on which during the next 2 weeks they grew into seedlings.

Torrenia fournieri. Pollen grains were introduced inside the ovaries through a slit in the ovary wall and later the whole ovaries were transferred on to the medium. Four days after pollination some ovules began enlarging and two weeks later they contained well differentiated embryos and endosperm. The torpedo embryos were then excised and cultured on MS medium. After the next 3 weeks of culture those embryos developed into small seedlings.

Chionodoxa luciliae. Ovules were pollinated one day following inoculation. Pollen grains germinated with high intensity and some of the fertilized ovules increased in size after only 3 days following pollination. Three weeks later they reached the stage of milk ripeness. After ten days the seeds became brown. Five weeks following pollination heart stage embryos were isolated and cultured on MS medium supplemented with 0.5 mg per l kinetin and 1 mg per l gibberellic acid. However, this medium did not support further development of the embryos.

INTERSPECIFIC AND INTERGENERIC POLLINATION OF OVULES

When ovules of *Primula pubescens* were pollinated with *P. auricula* the pollen grains started to germinate after 6-8 hours. During the first week of culture the fertilized ovules enlarged considerably and the unfertilized ones shrivelled (Fig. 7). A nuclear endosperm and a filamentous proembryo were found in sectioned 13 day-old ovules (Fig. 8). Four week after cross pollination the seeds still contained immature globular embryos which were not capable of further development.

Some ovules of *Scopolia carniolica* enlarged for a few days following pollination with pollen grains of *Physochlaina praealta*. Embryos and cellular endosperm were found in those analyzed 10 days later. After several more days seeds containing nearly mature embryos were formed. The embryos were excised and cultured on MS medium supplemented with 4 mg per l of 2,4-dichlorophenoxy acetic acid (2,4-D) and 2 mg per l of 6-benzyl aminopurine (BAP) on which they started to produce only calluses. During the next days of culture the calluses produced only roots (Fig. 9). Pollinated ovules of *Melandrium album* with pollen grains of *Silene saxifraga* and *Arenaria pungens* enlarged during the culture. The pollen of both species started to germinate within 8-12 hours and a few hours later the pollen tubes were entering the micropyle. During the first 3 days the fertilized ovules became turgid and in another 7 days they turned white. Globular embryos and nuclear endosperm were noticed in the 14 day-old seeds but they did not develop further and during the next weeks of culture they degenerated.

Fig. 1. 7 Day-old seedling of *Primula pubescens* obtained from self-pollinated ovules, $\times 6$. Fig. 2. Developing ovules (arrow) of *Primula auricula* situated on the placenta 6 days after self-pollination, $6 \times$. Fig. 3. Longitudinal sections of 21 day-old ovule of *Scopolia carniolica* after self-pollination, the presence of a dicotyledonous embryo and endosperm, $\times 224$. Fig. 4. 21 Day-old seedling of *S. carniolica* raised in culture from mature embryo, $\times 4$

Fig. 5. Longitudinal section of 27 day-old seed of *Digitalis purpurea* showing a cotyledonous embryo and a part of endosperm, $\times 540$. Fig. 6. Longitudinal section of 27 day-old seed of *D. purpurea* showing a globular embryo and a part of endosperm, $\times 560$. Figs. 7-8. *Primula pubescens* $\times$ *P. auricula*: Fig. 7—11 day-old culture showing several enlarged ovules (arrow) and small collapsing unfertilized ovules are also visible, $\times 4$. Fig. 8 — hybrid globular embryo and nuclear endosperm developed 13 days after cross-pollination, $\times 224$. Fig. 9. *Scopolia carniolica* $\times$ *Physoclaina praealta*; roots growing out from callus produced from the hybrid embryo, $\times 4$

DISCUSSION

Our results have shown that direct pollination of exposed ovules in ovaries make it possible to obtain viable seeds in 4 species. Embryos isolated from those seeds produced fully developed seedlings. In two other species (*Primula auricula* and *Chionodoxa luciliae*) seeds containing embryos at different stages of development were present but they did not grow further on the media used. It can be presumed that there are some disturbances, probably of a physiological nature inside the ovules which make the process of development and embryo growth impossible.

The main significance of the method of *in vitro* pollination of ovules is that it provides a means of bypassing the pollen/style rejection response present in self-incompatible or interspecifically incompatible plants. This method has been successfully used to bypass self-incompatibility in *Petunia axillaris* (Rangaswamy and Shivanna 1967) and in *Brassica campestris* (Zenkteler et al. 1987). Combining the techniques of *in vitro* fertilization and ovule or embryo culture has been successfully accomplished in 20 combinations of interspecific and intergeneric crosses (Zenkteler 1990). In 21 other combinations of crosses only globular hybrid embryos have been obtained. In our experiments on interspecific and intergeneric crosses we observed the formation of globular hybrid embryos during the first days following pollination. Usually those embryos developed until the second week of culture and later they started to degenerate. Promising results were obtained only when *Scopolia carniolica* was cross pollinated with *Physochlaina praealta*. In this combination of crosses, the embryos which reached a torpedo stage were isolated and transferred to a fresh medium on which they produced callus. It seems that the media which we used for culturing those embryos were not suitable, and therefore we were not able to obtain hybrid plants. Our knowledge about the nutritional requirements of immature globular hybrid embryos is very limited and at the moment there are no suitable methods for their culture. Embryos at the autotrophic phase of growth, which begins at the heart stage, can be cultured in media with ease as their requirements in artificial culture are less complex. Therefore, it is possible that, at least in the cross *Scopolia carniolica* × *Physochlaina praealta* we should be able to raise hybrid plants by providing more suitable media. Further experiments are needed to carry out investigations in this field of experimental embryology.

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Samozapylenie i zapylenie krzyżowe *in vitro* zalążków u niektórych gatunków

Streszczenie

Wykonano samozapylenie *in vitro* wyciętych łożysk wraz z zalążkami u następujących roślin: *Primula pubescens*, *P. auricula*, *Scopolia carniolica*, *Digitalis purpurea*, *Torenia fournieri* i *Chionodoxa luciliae*, a następnie obserwowano rozwój nasion. Stosując tę samą metodę otrzymano globularne zarodki hybrydowe z krzyżówek między: *P. pubescens* × *P. auricula*, *Scopolia carniolica* × *Physochlaina praealta*, *Melandrium album* × *Silene saxifraga* oraz *M. album* × *Arenaria pungens*.